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MICROSCOPY.[1]

On a New Method of Entrapping, Killing, Embedding and Orienting Infusoria and other very small Objects for the Microtome.—A reliable method for capturing, killing, staining and dehydrating minute organisms has long been a desideratum with biologists, especially when such objects fall far below 1–100th of an inch or 1–40th mm. in diameter. After trying a number of devices, all of which failed, I fortunately hit upon a plan that is not only very simple, but also capable of wide application, since I find that by its means organisms as small as 1–2000th inch or 12.5μ in diameter may be caught and held.

With the ordinary methods of filtration through the filter paper it is not possible to afterwards separate minute organisms thus captured from the surface of the substratum of paper. This difficulty has been overcome in my new method by means of a filter that *can* be easily cut in the block along with the objects adherent to its surface and within its meshes, a procedure quite impossible with filter paper. While filtration is the basis of this new method, and the use of ordinary filter paper is an essentially important part of it, it has been found necessary to find a substance that would serve as a filtering membrane that was porous, but not fibrous, because the presence of fibres is fatal to any attempt at cutting good sections in paraffine. On such a filtering membrane the organisms are caught and held where they are killed or fixed with any reagent, stained, dehydrated, and embedded, filter and all, in paraffine by means of the watch-glass method.

The filter upon which the objects are caught and killed consists of thin slices of elder pith. Get good, clean, whole pieces of elder pith, and clamp a piece of it into the holder of a Schanze or other sledge microtome, so as to make transverse sections of it, taking about four to six divisions of the micrometer wheel to each section. The microtome knife should be set at an acute angle with the line of movement of the knife carriage, as in cutting celloidin. With fresh pith, somewhat thinner sections may be cut. Upon examining such discoidal slices of elder pith, they will be found to be perforated at pretty regular intervals by openings caused by cutting through the very thin cellulose walls of certain of the pith cells. The pith filter is, it is thus seen, mechanically produced by the help of the microtome. A good supply of these little pith filters can be cut and kept in stock in a pill-box, ready for use at any time.

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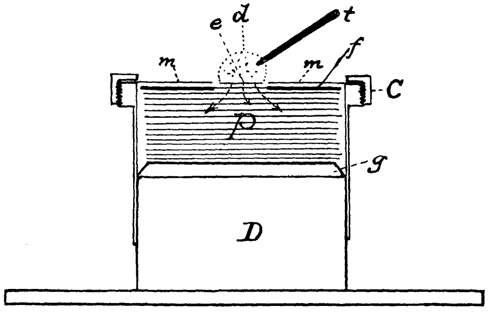
[1] Edited by C. O. Whitman, University of Chicago.

The next step is to take some ordinary good white filtering paper, cut into disks or squares about one inch in diameter. With a small drop of water on the end of a wooden toothpick, moisten a point at the centre of one of these paper disks or squares, so as to make a damp area just about the area of one of the disks of pith. Then take a wire 1–16 inch thick, heat one end, and with its help place on the paper disk or square some melted paraffine. This may be heated with the hot wire so as to saturate the whole of the paper disk or square, *except the central moistened spot,* which must be left unsaturated with paraffine.

The next step is to prepare a discoidal pad about one inch in diameter, composed of 10 to 20 superposed thicknesses of filter paper. Upon this the disk or square of filter paper, with all but the central spot saturated with paraffine, is superposed. I find that the ordinary extra large live-box or compressor, provided with a mica cover, with a round perforation of $\frac{5}{8}$ inch in diameter in its centre, is an excellent device for holding the disk prepared with paraffine down upon the thick pad of filter paper. The accompanying figure, showing the complete apparatus in vertical section, will perhaps make the arrangement of its parts clearer. The cap-ring *C* of the large-sized live box or compressor holds the perforated mica cover *m m* in place. This perforation should be a little larger than the disk of pith *e*, immediately below which lies the disk of filter paper *f*, which is saturated with paraffine except at its centre. Then follows the pad of several thicknesses of filter paper *P*. The mode of operation is as follows: Place *P* upon the glass disk *g* of the live box or compressor; then lay *f* upon *P*; then put the cap *C* with its centrally perforated mica cover *m m* in place and slip it



down over the drum *D*, so as to firmly hold *f* down upon *P*; then moisten the central exposed part of *f*, that is not saturated with paraffine, with a little water with a fine-nozzled pipette; then pick up one of the little disks of elder pith by one edge with a fine forceps and lay it down on the moist centre of *f*, with the convex side down, when it will at once flatten out and adhere to *f* and just neatly cover the central area not saturated with paraffine. The apparatus is now in readiness to begin operations.

Place a drop of water (*d*) swarming with animalcules from a vigorous culture of infusoria on *e*, when it will be found that the water will be rapidly drawn through *e* and *f* into *P* in the direction of the arrows. In this way several drops of water may have a large part of their animalcular population separated out and caught upon the surface of *e*. To kill the contents of *D* it is only necessary to add a little saturated corrosive solution or osmic acid, one per cent., with the help of a thin, slender wooden rod or toothpick to the drop. Enough of either of these killing and fixing agents can thus be added as a minute drop by simply thrusting the charged end of the wooden rod or toothpick *t* into the drop *d*. The animalcules are at once precipitated by the killing agents upon the upper surface of *e*, where, strange to say, they are caught and held in the meshes formed by the pith cells. The filter *e* may now be gently removed and lifted off *f* by means of a needle and forceps. With gentle handling I find that Ciliates will remain attached to *e*, and may be passed through a dozen reagents without their becoming detached, and that the pith disk, with its adherent objects, may be embedded in paraffine very readily by the watch-glass method.

Care should be exercised that the edges of the opening in *m m* do not come too close to the edge of *e*, else the water of the drop *d* will run off edgewise between *m* and *f*, and thus not pass through *e* alone. The paraffined portion of *f* should project a little beyond the free edges of *m*. Under such conditions the drop charged with organisms will round itself off as in the figure, and be kept from spreading by the greasy circular inner margin formed by the paraffine that saturates the margin of *f*. In lifting off *e*, raise its free edge slightly at one point with a needle; then catch the edge thus raised between the blades of a sharp-pointed forceps and transfer to a watch-glass containing 50 to 60 per cent. alcohol; then through the other solutions in succession.

Even orientation may be very easily effected by means of this method, either by sketching the outline of the whole disk and the position and direction of the axes of the very minute objects on it under a low power of the microscope, or else by shaving down the block after the disk of pith is embedded, so as to make it nearly transparent and so as to show the shape of the adherent organisms through the semitransparent block under a low power of the microscope. The proper cutting planes may now be marked or indicated on the margin of the block with lithographic ink or with a fine camel's-hair pencil with lampblack and turpentine. If the latter method is resorted to, great care must be exercised in scraping or shaving down the block to keep the plane along which the paraffine is removed parallel to the surface of the disk *e*.

I have found it very easy to thus capture, hold, kill, dehydrate, stain, embed and cut *Paramœcium aurelia*. *Euplotes*, *Stylonychia* and *Halteria* will also adhere to these disks. *Halteria* is about the size of a white blood-corpuscle, and the fact that it may be entrapped and treated as here described shows what a wide range of utility is promised by this new method of capturing and embedding minute organisms. It will doubtless also be found useful in the study of very minute eggs and larvæ.

I find that these pith disks, loaded with their adherent organisms, may be mounted entire, and one in this way may get most instructive preparations, often with half a dozen genera on a single slide. Staining is also entirely under control, and any of the usual stains or anilines or combinations of them may be successfully applied and the action watched under the microscope and arrested at just the proper moment. With this method it has been found possible to cut 18 longitudinal serial sections and 50 transverse serial sections of *Paramœcium* with a thickness of 2.5 to 5 μ with the Ryder Microtome set to 1 or 2 teeth of the micrometer wheel.

The fixation of the sections on the slide may be effected by means of Gustav Mann's albumen method. Take the white of an egg (30 c. c.), shake up with 300 c. c. of water for 5 minutes; filter twice. Paint clean slides on one side with this mixture with the aid of a glass rod, and stand up on end to drain and dry; 200 or more slides may be thus prepared and dried ready for use. The albumenized side of the dry sides may be distinguished by breathing upon them. The sections are to be stretched by floating the ribbon of paraffin containing them on warm water (30°c.). Immerse one end of the albumenized slide in the water and float and arrange the sections on it albumenized side uppermost. Place slide on water bath to dry, when the paraffin may be removed with xylol or turpentine after which the staining may be done on the slide. This method of fixing sections to slide with albumen is much simpler and more practical than with Meyer's formula.

These pith disks may also be used to introduce into the serous cavities of higher organisms and left long enough to become filled with entrapped leucocytes or lymph cells. I anticipate that such a method would greatly facilitate the study of karyokinesis and its technique of staining in connection with wandering cells in wounds.

The novelty and simplicity of this new method, as well as its wide range of applicability, impels me to promptly offer it to my fellow-naturalists as a procedure that will in many cases be found to materially facilitate their work, especially the work of those engaged in the study of Protozoa, or of very minute ova or larvæ. A very simple form of this apparatus, for holding the filtering paper in position, is being made and offered for sale by Chas. Lentz & Sons, of Philadelphia.

Dec. 9th, 1894.

JOHN A. RYDER.